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(54) Title: HUMAN ANTIBODIES DERIVED FROM IMMUNIZED XENOMICE

(57) Abstract

Antibodies with fully human variable regions against a specific antigen can be prepared by administering the antigen to a transgenic animal which has been modified to produce such antibodies in response to antigenic challenge, but whose endogenous loci have been disabled. Various subsequent manipulations can be performed to obtain either antibodies *per se* or analogs thereof.

Claims

1. A method to produce an immunoglobulin having fully human variable region or an analog thereof, specific for a desired antigen, which method comprises:

administering said antigen or an immunogenic portion thereof to a nonhuman animal under conditions to stimulate an immune response, whereby said animal produces B cells that secrete immunoglobulin specific for said antigen; wherein said nonhuman animal is characterized by being substantially incapable of producing endogenous heavy and light immunoglobulin chain variable regions, but capable of producing human immunoglobulin variable regions; and

recovering said immunoglobulin or analog.

2. The method of claim 1 wherein said recovering step comprises recovering polyclonal immunoglobulin or analog from said animal.

3. The method of claim 1 wherein said recovering step comprises immortalizing B cells from said animal immunized with said antigen, screening the resulting immortalized cells for the secretion of said immunoglobulin specific for said antigen, and

1) recovering immunoglobulin secreted by said immortalized B cells, or

2) recovering the genes encoding at least the variable region of said immunoglobulin from the immortalized B cells, and optionally modifying said genes;

expressing said genes or modified forms thereof to produce immunoglobulin or analog; and

recovering said immunoglobulin or analog.

4. The method of claim 1 wherein said recovering step comprises

recovering genes encoding at least the variable region of immunoglobulins from the primary B cells of the animal immunized with said antigen;

generating a library of said genes expressing the variable regions;

screening the library for a variable region with desired affinity for the antigen;

recovering the genes encoding said variable regions and optionally modifying said genes;

expressing said recovered genes to produce an immunoglobulin or analog containing said variable region and recovering said immunoglobulin or analog.

5. The method of claim 1 wherein said immunoglobulin is fully human.

6. A recombinant DNA molecule comprising a nucleotide sequence encoding the immunoglobulin or analog produced by the method of claim 1.

7. A recombinant DNA molecule comprising an encoding nucleotide sequence corresponding to a gene prepared by a method comprising

administering a desired antigen or an immunogenic portion thereof to a nonhuman animal under conditions to stimulate an immune response, whereby said animal produces B cells that secrete immunoglobulin specific for said antigen; wherein said nonhuman animal is characterized by being substantially incapable of producing endogenous heavy and light immunoglobulin chain variable regions, but capable of producing human immunoglobulin variable regions;

immortalizing B cells from said animal immunized with said antigen, screening the resulting immortalized cells for the secretion of said immunoglobulin specific for said antigen, and

recovering the genes encoding at least the variable region of said immunoglobulin from the immortalized B cells, and optionally modifying said genes.

8. A recombinant DNA molecule comprising an encoding nucleotide sequence corresponding to a gene prepared by a method comprising

administering a desired antigen or an immunogenic portion thereof to a nonhuman animal under conditions to stimulate an immune response, whereby said animal produces B cells that secrete immunoglobulin specific for said antigen; wherein said nonhuman animal is characterized by being substantially incapable of producing endogenous heavy and light immunoglobulin chain variable regions, but capable of producing human immunoglobulin variable regions;

recovering genes encoding at least the variable region of immunoglobulins from the primary B cells of the animal immunized with said antigen;

generating a library of said genes expressing the variable regions;

screening the library for a variable region with desired affinity for the antigen; and

recovering the genes encoding said variable regions and optionally modifying said genes.

9. The DNA molecule of claim 6, 7 or 8 wherein said encoding nucleotide sequence is operably linked to control sequences capable of effecting its expression.

10. A cell or cell line modified to contain the DNA molecule of claim 9.

11. A method to produce an immunoglobulin with fully human variable region or an analog thereof which method comprises culturing the cells of claim 10 under conditions whereby said encoding nucleotide sequence is expressed to produce said immunoglobulin or analog; and

recovering said immunoglobulin or analog.

12. An immortalized B cell which secretes an immunoglobulin with a fully human variable region to a desired antigen prepared by a method which comprises

administering said antigen or an immunogenic portion thereof to a nonhuman animal under conditions to stimulate an immune response, whereby said animal produces B cells that

secrete immunoglobulin specific for said antigen; wherein said nonhuman animal is characterized by being substantially incapable of producing endogenous heavy and light immunoglobulin chain variable regions, but capable of producing human immunoglobulin variable regions;

immortalizing B cells from said animal immunized with said antigen, screening the resulting immortalized cells for the secretion of said immunoglobulin specific for said antigen; and

recovering said immortalized B cell.

13. A method to produce an immunoglobulin or analog which comprises culturing the recovered cells of claim 12 and recovering said immunoglobulin or analog.

14. An immunoglobulin with fully human variable region or analog thereof produced by the method of claim 1.

15. The immunoglobulin or analog of claim 14 which is fully human.

16. The immunoglobulin or analog of claim 14 which is an agonist or a catalyst or wherein the immunoglobulin is chimeric.

17. The immunoglobulin or analog of claim 14 wherein the desired antigen is selected from the group consisting of transition state mimics; leukocyte markers; histocompatibility antigens; adhesion molecules; interleukins; interleukin receptors; chemokines; growth factors; growth factor receptors; interferon receptors; Igs and their receptors; tumor antigens; allergens; viral proteins; toxins; blood factors; enzymes; and the miscellaneous antigens ganglioside GD3, ganglioside GM2, LMP1, LMP2, eosinophil major basic protein, eosinophil cationic protein, pANCA, Amadori protein, Type IV collagen, glycated lipids, γ-interferon, A7, P-glycoprotein, Fas (AFO-1) and oxidized-LDL.

18. The immunoglobulin or analog of claim 17 wherein the leukocyte marker is selected from the group consisting of CD2, CD3, CD4, CD5, CD6, CD7, CD8, CD11a,b,c, CD13, CD14, CD18, CD19, CD20, CD22, CD23, CD27 and its ligand, CD28 and its ligands B7.1, B7.2, B7.3, CD29 and its ligand, CD30 and its ligand, CD40 and its ligand gp39, CD44, CD45 and isoforms, CDw52 (Campath antigen), CD56, CD58, CD69, CD72, CTLA-4, LFA-1 and TCR;

the histocompatibility antigen is selected from the group consisting of MHC class I or II, the Lewis Y antigens, SLex, SLey, SLea, and SLeb;

the adhesion molecule is selected from the group consisting of VLA-1, VLA-2, VLA-3, VLA-4, VLA-5, VLA-6, LFA-1, L-selectin, P-selectin, and E-selectin and their counterreceptors VCAM-1, ICAM-1, ICAM-2, LFA-3; Mac-1 and p150,95;

the interleukin is selected from the group consisting of IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, and IL-15;

the interleukin receptor is selected from the group consisting of IL-1R, IL-2R, IL-3R, IL-4R, IL-5R, IL-6R, IL-7R, IL-8R, IL-9R, IL-10R, IL-11R, IL-12R, IL-13R, IL-14R, and IL-15R;

the chemokine is selected from the group consisting of PF4, RANTES, MIP1α, MCP1, NAP-2, Groα, Groβ, and IL-8;

the growth factor is selected from the group consisting of TNFalpha, TGFbeta, TSH, VEGF/VPF, PTHrP, EGF family, FGF, PDGF family, endothelin, and gastrin releasing peptide (GRP);

the growth factor receptor is selected from the group consisting of TNFalphaR, RGFbetaR, TSHR, VEGFR/VPFR, FGFR, EGFR, PTHrPR, PDGFR family, EPO-R, GCSF-R and other hematopoietic receptors;

the interferon receptor is selected from the group consisting of IFNαR, IFNβR, and IFNγR;

the Ig and its receptor is selected from the group consisting of IgE, FceRI, and FCeRII;

the tumor antigen is selected from the group consisting of her2-neu, mucin, CEA and endosialin;

the allergen is selected from the group consisting of house dust mite antigen, lol p1 (grass) antigens, and urushiol;

the viral protein is selected from the group consisting of CMV glycoproteins B, H, and gCIII, HIV-1 envelope glycoproteins, RSV envelope glycoproteins, HSV envelope glycoproteins, EBV envelope glycoproteins, VZV envelope glycoproteins, HPV envelope glycoproteins, Hepatitis family surface antigens;

the toxin is selected from the group consisting of pseudomonas endotoxin and osteopontin/uropontin, snake venom, and bee venom;

the blood factor is selected from the group consisting of complement C3b, complement C5a, complement C5b-9, Rh factor, fibrinogen, fibrin, and myelin associated growth inhibitor; and

the enzyme is selected from the group consisting of cholesterol ester transfer protein, membrane bound matrix metalloproteases, and glutamic acid decarboxylase (GAD).

19. The immunoglobulin or analog of claim 14 wherein said desired antigen is selected from the group consisting of human IL-6, human IL-8, human TNFα, human CD4, human L-selectin, human gp39, human IgE and tetanus toxin C(TTC).

20. A recombinant DNA molecule comprising a nucleotide sequence that encodes the immunoglobulin or analog of any of claims 15-19.

21. The DNA molecule of claim 20 wherein said encoding nucleotide sequence is operably linked to control sequences capable of effecting its expression.

22. A cell or cell line modified to contain the DNA molecule of claim 21.

23. A method to produce an immunoglobulin or analog specific for a desired antigen which method comprises culturing

the cell or cell line of claim 22 under conditions wherein said nucleotide sequence is expressed to produce said immunoglobulin or analog; and

recovering the immunoglobulin or analog.

24. An antibody containing a fully human variable region or analog thereof which is specifically immunoreactive with an antigen selected from the group consisting of transition state mimics; leukocyte markers; histocompatibility antigens; adhesion molecules; interleukins; interleukin receptors; chemokines; growth factors; growth factor receptors; interferon receptors; Igs and their receptors; tumor antigens; allergens; viral proteins; toxins; blood factors; enzymes; and the miscellaneous antigens ganglioside GD3, ganglioside GM2, LMP1, LMP2, eosinophil major basic protein, eosinophil cationic protein, pANCA, Amadori protein, Type IV collagen, glycated lipids, γ-interferon, A7, P-glycoprotein, Fas (AFO-1) and oxidized-LDL.

25. The antibody or analog of claim 24 wherein the leukocyte marker is selected from the group consisting of CD2, CD3, CD4, CD5, CD6, CD7, CD8, CD11a,b,c, CD13, CD14, CD18, CD19, CD20, CD22, CD23, CD27 and its ligand, CD28 and its ligands B7.1, B7.2, B7.3, CD29 and its ligand, CD30 and its ligand, CD40 and its ligand gp39, CD44, CD45 and isoforms, CDw52 (Campath antigen), CD56, CD58, CD69, CD72, CTLA-4, LFA-1 and TCR;

the histocompatibility antigen is selected from the group consisting of MHC class I or II, the Lewis Y antigens, SLex, SLey, SLea, and SLeb;

the adhesion molecule is selected from the group consisting of VLA-1, VLA-2, VLA-3, VLA-4, VLA-5, VLA-6, LFA-1, L-selectin, P-selectin, and E-selectin and their counterreceptors VCAM-1, ICAM-1, ICAM-2, LFA-3; Mac-1 and p150,95;

the interleukin is selected from the group consisting of IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, and IL-15;

the interleukin receptor is selected from the group consisting of IL-1R, IL-2R, IL-3R, IL-4R, IL-5R, IL-6R, IL-7R, IL-8R, IL-9R, IL-10R, IL-11R, IL-12R, IL-13R, IL-14R, and IL-15R;

the chemokine is selected from the group consisting of PF4, RANTES, MIP1α, MCP1, NAP-2, Groα, Groβ, and IL-8;

the growth factor is selected from the group consisting of TNFalpha, TGFbeta, TSH, VEGF/VPF, PTHrP, EGF family, FGF, PDGF family, endothelin, and gastrin releasing peptide (GRP);

the growth factor receptor is selected from the group consisting of TNFalphaR, RGFbetaR, TSHR, VEGFR/VPFR, FGFR, EGFR, PTHrPR, PDGFR family, EPO-R, GCSF-R and other hematopoietic receptors;

the interferon receptor is selected from the group consisting of IFNαR, IFNβR, and IFNγR;

the Ig and its receptor is selected from the group consisting of IgE, FceRI, and FCeRII;

the tumor antigen is selected from the group consisting of her2-neu, mucin, CEA and endosialin;

the allergen is selected from the group consisting of house dust mite antigen, lol p1 (grass) antigens, and urushiol;

the viral protein is selected from the group consisting of CMV glycoproteins B, H, and gCIII, HIV-1 envelope glycoproteins, RSV envelope glycoproteins, HSV envelope glycoproteins, EBV envelope glycoproteins, VZV envelope glycoproteins, HPV envelope glycoproteins, Hepatitis family surface antigens;

the toxin is selected from the group consisting of pseudomonas endotoxin and osteopontin/uropontin, snake venom, and bee venom;

the blood factor is selected from the group consisting of complement C3b, complement C5a, complement C5b-9, Rh factor, fibrinogen, fibrin, and myelin associated growth inhibitor; and

the enzyme is selected from the group consisting of cholesterol ester transfer protein, membrane bound matrix metalloproteases, and glutamic acid decarboxylase (GAD).

26. The antibody or analog of claim 24 wherein the desired antigen is selected from the group consisting of human IL-6, human IL-8, human TNFα, human CD4, human L-selectin, human gp39, human IgE and tetanus toxin C(TTC).

27. The antibody or analog of claim 19 or 26 wherein the desired antigen is human IL-6.

28. The antibody or analog of claim 19 or 26 wherein the desired antigen is human IL-8.

29. The antibody or analog of claim 19 or 26 wherein the desired antigen is human TNFα.

30. The antibody or analog of claim 19 or 26 wherein the desired antigen is human CD4.

31. The antibody or analog of claim 19 or 26 wherein the desired antigen is human L-selectin.

32. The antibody or analog of claim 19 or 26 wherein the desired antigen is human gp39.

33. The antibody or analog of claim 19 or 26 wherein the desired antigen is tetanus toxin C(TTC).

34. The antibody or analog of claim 19 or 26 wherein the desired antigen is human IgE.

35. The analog of claim 19 or 26 which is a single chain F_V.

36. The antibody or analog of claim 24 which is fully human.

37. The antibody or analog of claim 24 which is an agonist or is a catalyst or wherein the immunoglobulin is chimeric.

38. A recombinant DNA molecule encoding the antibody or analog of any of claims 26-37.

39. A recombinant DNA molecule which comprises an expression system for the production of the antibody or analog of any of claims 26-37 which expression system comprises a nucleotide sequence encoding said antibody or analog operably linked to control sequences capable of effecting its expression.

40. A recombinant host cell which is modified to contain the DNA molecule of claim 39.

41. A method to produce an antibody or analog which method comprises culturing the cells of claim 40 under conditions wherein said coding sequence is expressed; and recovering the antibody or analog produced.

42. Use of the antibody or analog of claim 36 for *in vivo* prophylaxis, therapy or diagnosis in humans.

43. Use of the antibody or analog of claim 27, 29, 30, 31 or 32 for treating an autoimmune disease in a mammal.

44. The use of claim 43 wherein the autoimmune disease is systemic lupus erythrematosus, rheumatoid arthritis, psoriasis, Sjogren's syndrome, scleroderma, mixed connective tissue disease, dermatomyositis, polymyositis, Reiter's syndrome, Behcet's disease, Type I diabetes, Hashimoto's thyroiditis, Graves' disease, multiple sclerosis, myasthenia gravis, or pemphigus.

45. Use of the antibody of claim 32 for preventing graft versus host disease, for preventing rejection of an organ transplant, or for treating glomerular nephritis in a mammal.

46. Use of the antibody of claim 31 for treating reperfusion ischemia in a mammal.

47. Use of the antibody of claim 27 for treating cachexia, septic shock, myeloma, renal cell carcinoma, osteoporosis, or Paget disease in a mammal.

48. Use of the antibody of claim 29 for treating septic shock, cachexia, osteoporosis, or systemic sclerosis in a mammal.

49. Use of the antibody of claim 28 for preventing tumor metastasis, and for treating asthma, rheumatoid arthritis, glomerulonephritis, reperfusion injury, adult respiratory distress syndrome, or systemic sclerosis in a mammal.